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Letters to the Editor

Resistant Hypertriglyceridemia in a Patient With High Plasma Levels of Apolipoprotein CII

To the Editor:

Human apolipoprotein CII (apo CII) consists of 79 amino acid residues and is required as a cofactor in the hydrolysis of triacylglycerides of chylomicrons and VLDL by lipoprotein lipase.[1,2] Familial apo CII deficiency is an autosomal recessive genetic disorder characterized by fasting hypertriglyceridemia and an accumulation of chylomicrons in the plasma.[3] Shachter et al[4] generated transgenic mice overexpressing human apo CII, and these authors reported the unexpected observation of marked hypertriglyceridemia with an accumulation of triglyceride-enriched VLDL in the plasma. We are the first to report a case of resistant hypertriglyceridemia in a young man with high plasma levels of apo CII (turbidimetric method by Alpha-Biotech, Milan, Italy).

A 42-year-old white man was referred to our lipid clinic for diet- and drug-resistant hypertriglyceridemia. His familial history was positive for cardiovascular diseases (father with hypercholesterolemia and myocardial infarction). He had stopped smoking 9 years ago. He presented a history of chest pain, but the baseline ECG and a strength test were normal. He also underwent an ultrasound scan of the abdomen, which showed normal morphology of the liver and gallbladder. His blood pressure was normal (120/80 mm Hg), and he usually performed adequate physical activity. The Table shows the lipid profile of the patient at the first visit, after 1 month of diet therapy (1800 kcal/d and total abstention from alcohol consumption), and after 1 month of diet plus 400 mg of fenofibrate. We also treated the patient with 1200 mg×3/d of gemfibrozil without changes in the lipid profile. No floating chylomicrons were detected in the plasma sample after 24 hours at 4.0°C, although the plasma remained turbid. The results of laboratory tests included a fasting plasma glucose of 82 mg/dL, an alanine aminotransferase level of 23 IU/L, an aspartate aminotransferase of 13 IU/L, and a γ-glutamyl transpeptidase of 30 IU/L. Considering the lack of results with drug therapy, the patient is currently being treated with diet only.

Lipid Profile of a Patient With Resistant Hypertriglyceridemia at Baseline, 1 Month After Diet Therapy (1800 kcal and Total Abstention From Alcohol Consumption), 1 Month After Diet and 400 mg of Fenofibrate, and 1 Month After Diet and 3600 mg of Gemfibrozil

	Baseline	Diet 1800 of kcal/d	Diet+ 400 mg Fenofibrate	Diet+ 3600 mg Gemfibrozil
Body mass index, kg/m^2	23	19.8	20	19.9
Triglycerides, mg/dL	550	451	485	456
Total cholesterol, mg/dL	190	146	195	182
HDL cholesterol, mg/dL	45	49	67	54
HDL2 cholesterol, mg/dL	16	13	18	14
HDL3 cholesterol, mg/dL	29	36	49	40
Apo AI, mg/dL	119	126	109	114
Apo B, mg/dL	106	100	105	121
Apo CII, mg/dL	15.4	15.6	14.8	15.9 (11.2 after removal of TRL)
Apo CIII, mg/dL	6.0	7.2	6.3	6.2 (6.1 after removal of TRL)
Lipoprotein(a), mg/dL	44.9	45.0	38.6	42.6
APOE genotype	E3/E3			

TRL indicates triglyceride-rich lipoproteins. Apolipoproteins CII and CIII were measured in total plasma and after ultracentrifugation to remove VLDL and chylomicrons (triglyceride-VLDL after ultracentrifugation was raised to 1000 mg/dL). The normal range in adult males for apo CII is: 1.0–5.5 mg/dL; for apo CIII, 6.0–12.0 mg/dL.

The molecular mechanisms of hypertriglyceridemia are not well understood; however, it is well known that apo CII stimulates lipoprotein lipase. The possibility that the high plasma levels of triglycerides described in this case were related to impaired remnant particle removal could be ruled out, considering the normal plasma cholesterol levels; we can argue that the defect could be in the lipolysis. Furthermore, it has been shown that high levels of apo CII directly inhibit lipoprotein lipase[5] and that a high level of human apo CII is inhibitory to mouse lipoprotein lipase.[4] It has been considered that an excess of apo CII may impair lipolysis by decreasing the access of lipoprotein particles to lipases; in fact, apo CII has been shown to decrease the association of lipoprotein lipase with phospholipid vesicles, and thus, excess apo CII may interfere with the association of triglyceride-rich lipoproteins with glycosaminoglycans, thereby impairing both lipolysis and particle clearance.[4] This case raises the possibility that overexpression of apo CII could have a different role in the catabolism of triglyceride-rich lipoproteins, leading to increased levels of several atherogenic species, including cholesterol-enriched VLDL.

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Oxidized LDL Can Promote Human Monocyte Survival

To the Editor:

It is likely that in the early stages of atherosclerosis, circulating monocytes migrate into the subendothelial space, where they can mature into foam cells.[1–5] There is in vivo and in vitro evidence for both foam cell death but also enhanced survival and growth.[6–22]

Human peripheral blood monocytes (≥95% pure) were obtained by countercurrent elutriation and usually cultured in minimal essential medium, α-modification (α-MEM)/1% pooled normal human serum (HS).[23,24] The number of viable cells was measured by scraping the tissue culture surface and counting them in a hemocytometer with trypan blue exclusion or by propidium iodide staining (flow cytometry). Oxidized LDL (ox-LDL) was prepared as before.[14]

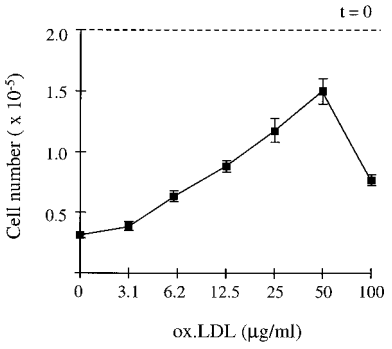

Figure I. Effect of ox-LDL dose on human monocyte survival. Elutriation-purified human monocytes were plated at 2×10^5 monocytes (t=0) in α-MEM/1% HS and were either left untreated or treated with increasing concentrations of ox-LDL. After 5 days, viable cell number was determined (hemocytometer, trypan blue exclusion). Data are from a representative experiment, which was repeated 12 lines with monocytes from different donors, and are mean values+SEM from triplicate cultures.

The number of viable monocytes declined when they were left untreated or treated with native LDL; this loss was reduced by both ox-LDL and acetylated LDL (ac-LDL; see the Table). A dose response for the ox-LDL effect is provided in the online Figure (please see http://atvb.ahajournals.org) and, as we found before with murine macrophages,[14] doses of ox-LDL $\leq$50 μg/mL generally promoted survival; at these survival-inducing doses, the cells spread on the tissue culture surface and remained attached. In contrast, at higher concentrations, viable cell numbers again declined. With different ox-LDL preparations, the effective survival dose response varied to some extent. The ability of ox-LDL to enhance human monocyte survival was confirmed with monocytes from 30 donors. We previously found that prior adherence of the monocytes for a short period under serum-free conditions, followed by culture in 1% HS, improved the subsequent viability of the cells.[24] Under these conditions, ox-LDL was able to maintain the original cell number (online Table I; please see http://atvb.ahajournals.org).

It is possible that the enhanced human monocyte survival by ox-LDL described above is due to endogenous granulocyte macrophage–colony stimulating factor (GM-CSF) and/or CSF-1.[25–27] For ox-LDL–treated cultures, no evidence could be found for a requirement for either CSF by using blocking monoclonal antibodies to the ligands and to the CSF-1 receptor (online Tables II and III; please see http://atvb.ahajournals.org). For most experiments, the antibodies reduced the number of viable cells in the untreated cultures, suggesting that endogenous GM-CSF and CSF-1 play a role in monocyte survival in 1% HS (online Tables II and III); this inhibitory effect on basal survival led, in some experiments, to an apparent reduction in the number of viable cells in the ox-LDL–treated cultures, which could, however, be accounted for by an effect on the survival of the non–ox-LDL–treated cells (data not shown).

TABLE II. Effect of Antibody to GM-CSF on Ox-LDL–Induced Human Monocyte Survival

	Cell No., $\times10^{-5}$	
Treatment	$-\alpha$-GM-CSF	$+\alpha$-GM-CSF
...	0.23±0.03	0.12±0.02
Ox-LDL	0.80±0.04	0.79±0.03
GM-CSF	0.78±0.03	0.40±0.04

Elutriation-purified human monocytes were plated at 1.5×10^5 monocytes in α-MEM/1% HS and were either left untreated or treated with ox-LDL (50 μg/mL) or GM-CSF (100 U/mL), in the absence (−) or presence (+) of anti–GM-CSF antibody (15 μg/mL). After 5 days, viable cell number was determined. Data are provided from a representative experiment. The experiments were repeated another 12 times with different monocyte populations.

Effect of Ox-LDL and Ac-LDL on Human Monocyte Survival

Treatment	Cell No., $\times10^{-5}$
...	0.23±0.02
LDL	0.24±0.02
Ox-LDL	1.1±0.1
Ac-LDL	1.0±0.1

Elutriation-purified human monocytes were plated at 2×10^5 cells in α-MEM/1% HS and were either left untreated or treated with 50 μg/mL LDL, ox-LDL, or ac-LDL. After 5 days, viable cell number was determined (hemocytometer, trypan blue exclusion). Data are from a representative experiment, which was repeated 18 times with cells from different donors, and are mean values±SEM from triplicate cultures.

Prior studies have found that ox-LDL caused apoptosis in adherence-prepared human monocyte cultures.[12] However, in that study, only ox-LDL concentrations $\geq$50 μg/mL were examined, and the toxic response increased as the concentration of the lipoprotein was raised to 200 μg/mL; the effects of lower concentrations were not reported. From our studies, it is important to titrate the concentration of each ox-LDL batch on human monocytes. Our findings on the reversal of cell death by ox-LDL are similar to what we have published previously with murine macrophages.[14] Others have found that human macrophages, derived after maturation from 9-day cultures of monocytes, subsequently showed a proliferative response when treated with 10 to 50 μg/mL ox-LDL.[5] We found no evidence of increased DNA synthesis (tritiated thymidine incorporation) over the 5-day period in our ox-LDL–treated human monocytes (data not shown).

The few studies that have measured the amounts of oxidation products, eg, oxysterols, present in foam cells from human lesions have found them to be small[28]; also during the early stages of atherosclerosis, the amount of ox-LDL is likely to be low. It could therefore be argued that lower ox-LDL loadings could more likely better represent the in vivo situation than the high (toxic) levels, although it could be imagined that at more advanced stages of the disease, increased accumulation of ox-LDL may generate a toxic effect.[29] Our data could help explain both the increased numbers of foam cells, as well as the presence of apoptotic cells, in atheroma (see also Reference 14).

We have demonstrated above that ac-LDL was quite potent in promoting human monocyte survival. Uptake of ox-LDL by macrophages occurs in part through the ac-LDL receptor,[30,31] but several lines of evidence point to the existence of a number of receptors for

TABLE III. Effect of Antibodies to CSF-1 and to Its Receptor on Ox-LDL–Induced Human Monocyte Survival

	Cell No., $\times10^{-5}$		
Treatment	No Antibody	$+\alpha$-CSF-1	$+\alpha$-CSF-1R
...	0.23±0.03	0.21±0.03	0.06±0.01
Ox-LDL	0.80±0.04	0.89±0.04	0.77±0.05
CSF-1	0.78±0.04	0.45±0.03	0.20±0.03

Elutriation-purified monocytes were plated at 1.5×10^5 monocytes in α-MEM/1% HS and were either left untreated or treated with ox-LDL (50 μg/mL) or CSF-1 (1250 U/mL), in the absence or presence of anti–CSF-1 antibody (2 μg/mL) or anti–CSF-1 receptor (R) antibody (50 ng/mL). After 5 days, viable cell number was determined. Data are provided from a representative experiment with monocytes from different donors and are mean values±SEM from triplicate cultures. The experiment was performed with the same cells that were used in the experiment of Table II. The experiment was repeated another 14 times with different monocyte populations.

ox-LDL.[32] The contribution of different receptor usage to the effects on human monocyte survival remains to be elucidated. Our result with ac-LDL and human monocytes is consistent with our findings in murine macrophages;[14] in contrast, others have distinguished ac-LDL from ox-LDL by the inability of the former to induce murine macrophage growth.[33]

In summary, foam cells in atherosclerotic plaques are widely believed to result from the uptake by monocytes/macrophages of LDL after its modification, eg, by oxidation. Human monocytes slowly die in vitro, an apoptotic process that has been reported to be enhanced after addition of ox-LDL.[12] We report here that the effect of ox-LDL on the survival of elutriation-purified human monocytes in vitro is dose dependent, with high concentrations being toxic but lower concentrations in fact promoting survival. Ac-LDL, but not native LDL, was also active in enhancing monocyte survival. Addition of blocking monoclonal antibodies to either GM-CSF or CSF-1 failed to provide evidence for an essential role for these CSFs in ox-LDL–promoted monocyte survival. The data could help explain both the increased numbers of foam cells, as well as the presence of apoptotic cells, in atheroma.

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